

The role of fixation in the diagnosis of disease using spectral cytopathology

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Spectral Cytopathology (SCP)¹ is an innovative approach for disease diagnosis that utilizes infrared micro-spectroscopy (IR-MSP) to interrogate unstained tissue or cellular samples and analyzes spectral data using unsupervised multivariate statistical methods, such as principal component analysis (PCA).² In the past decade, SCP has taken considerable strides in its application for disease diagnosis; however, the effects of sample fixation and storage are still not thoroughly understood and often debated.^{3,4} Conversely, fixation and staining methods in traditional cytopathology, typically focused on maintaining the morphology of cells, have been documented and widely accepted for nearly a century. For SCP, fixation procedures must preserve the sample's biochemical composition so that spectral changes significant to disease diagnosis are not masked.

We report efforts to study the effects of fixation methods commonly used in traditional cytopathology and SCP to demonstrate its role in disease diagnosis. Both fixed (*i.e.* buffered formalin and alcohol mixture solutions) and unfixed protocols applied to exfoliated and cultured cells are presented to understand if changes differ based on cell type or fixation protocol. Data suggest that length of time in fixative and duration of sample storage *via* desiccation contribute to minor spectral changes, where spectra are nearly super-imposable. Conclusions imply that changes due to fixation are negligible in comparison to changes induced by disease.

References

- [1] J.M. Schubert, A.I. Mazur, B. Bird, M. Miljković, M. Diem, *J Biophoton* **3**, 588-596 (2010).
- [2] S. Boydston-White, M. Romeo, T. Chernenko, A. Regina, M. Miljković, M. Diem, *Biochim Biophys Acta* **1758**, 908-914 (2006).
- [3] H.H. Mantsch, M. Jackson, *J Mol Struct* **347**, 187-206 (1995).
- [4] G. Hastings, R. Wang, P. Krug, D. Katz, J. Hilliard, *Biopolymers* **89**, 921-930 (2008).